REPORT DOCUMENTATION PAGE

Form Approved
OMB NO. 0704-0188

1. AGENCY USE ONLY (Leave Blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	28-JUN-2001	FINAL REPORT 01 AUG,1997 – 31 MAY, 2001

4. TITLE AND SUBTITLE
Detection of Biological and Non-Biological Aerosols via Fluorescence and Surfactant on Droplets via Harmonic Generations

5. FUNDING NUMBERS
DAAG55-97-1-0349

6. AUTHOR(S)
Richard K. Chang

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Department of Applied Physics
Yale University
P.O. Box 208284
New Haven, CT 06520

20011024 050

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U. S. Army Research Office
P.O. Box 12211
Research Triangle Park, NC 27709-2211

10. SPONSORING / MONITORING AGENCY REPORT NUMBER
36605.12-EV

11. SUPPLEMENTARY NOTES
The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision, unless so designated by other documentation.

12 a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for public release; distribution unlimited.

12 b. DISTRIBUTION CODE

13. ABSTRACT (Maximum 200 words)

For the entire grant period, the objective has been to use optical diagnostic techniques to characterize the airborne particles. Ideally, the particles in the ambient, with or without deliberate disbursement, need to be classified and, if possible, be identified. Conditioned on the classification of particles, that randomly transit through the sample volume, the particles would or would not be deflected to another location where the particles would be subject to series of biochemical-related identification. Our challenge is to develop optical techniques that could serve as the preliminary screener of every airborne particle that randomly transits (at 1-10 m/s) through the sample volume. The challenge is to discriminate the bio- from the non-bio-aerosols within a time short enough to trigger the particle deflector and possibly to set off an alert alarm.

14. SUBJECT TERMS
fluorescence, elastic scattering, biological particles, tryptophan riboflavin, lumichrome, and droplets

15. NUMBER OF PAGES
59

16. PRICE CODE

17. SECURITY CLASSIFICATION OR REPORT	18. SECURITY CLASSIFICATION ON THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
UNCLASSIFIED	UNCLASSIFIED	UNCLASSIFIED	UL

NSN 7540-01-280-5500

Standard Form 298 (Rev.2-89)
Prescribed by ANSI Std. 239-18
298-102

DETECTION OF BIOLOGICAL AND NON-BIOLOGICAL AREOSOLS VIA FLUORESCENCE AND SURFACTANT ON DROPLETS VIA HARMONIC GENERATIONS

FINAL REPORT

RICHARD K. CHANG

01 AUG,1997 – 31, MAY, 2001

US ARMY RESEARCH OFFICE

DAAG5-97-1-0349

YALE UNIVERSITY

STATEMENT OF THE PROBLEM STUDIED:

For the entire grant period, the objective has been to use optical diagnostic techniques to characterize the airborne particles. Ideally, the particles in the ambient, with or without deliberate disbursement, need to be classified and, if possible, be identified. Conditioned on the classification of particles, that randomly transit through the sample volume, the particles would or would not be deflected to another location where the particles would be subject to series of biochemical-related identification. Our challenge is to develop optical techniques that could serve as the preliminary screener of every airborne particle that randomly transits (at 1-10 m/s) through the sample volume. The challenge is to discriminate the bio- from the non-bio-aerosols within a time short enough to trigger the particle deflector and possibly to set off an alert alarm.

SUMMARY OF THE MOST IMPORTANT RESULTS

During the period of this grant several important results were achieved which can be briefly summarized into three parts: For more details on each part, please refer to the publications (see the list of all publications).

1) We were able to detect the entire fluorescence spectra (from 250 nm to 700 nm) from a near-unity fraction of ambient aerosols at a reasonably large drawing volume rate (500 liters/min.) into an airtight box. In our experiments, the realization that a surprisingly high number of aerosols exiting the input nozzle (2 mm ID) is detected suggests that our specially designed nozzle was partially "focusing" the aerosols in the vicinity of the laser-sample volume. It is most rewarding to make the time investment necessary to design a nozzle that can focus the aerosols into a volume comparable to the laser-sample volume 50x50x50 μm^3. With a 2 mm (ID) entrance nozzle, focusing of aerosols into the laser focal volume would increase the particle diagnostic rate by $(2000/50)^2 = 1{,}600X$. By a shadowgraph- imaging technique we know the aerosol-focusing waist to be 0.5 mm and, thus, we have achieved a focusing enhancement of $(2000/500)^2 = 16X$. Nevertheless, the present instrument can detect the presence of a dog in the laboratory, when people are moving around and talking in the laboratory, and the room dust settles when the laboratory is empty (see Figs. 1 and 2).

2) We introduced a new technique to record the angular distribution, within a $\pm 20^\circ$ range of polar angles (θ) and equatorial angles(ϕ), of the elastic scattering from any individual aerosols. By using the Abbe sine condition, a perfect lens transform a given pair of angles (θ,ϕ) into an unique point (x,y) in a plane of a CCD camera. We coined this technique with an acronym TAOS to represent two-dimensional angular optical scattering. TAOS has been determined for spherical, spheroidal (oblate and prolate), and a pair of nearly touching microdroplets as well as clusters of mono-dispersed polystyrene latex spheres and *B. Subtillis*. We have interested several world-class theorists (e.g., G. Videen, O.I. Sindoni, and S.C. Hill) to compare their computer-intensive calculations of spheroids and

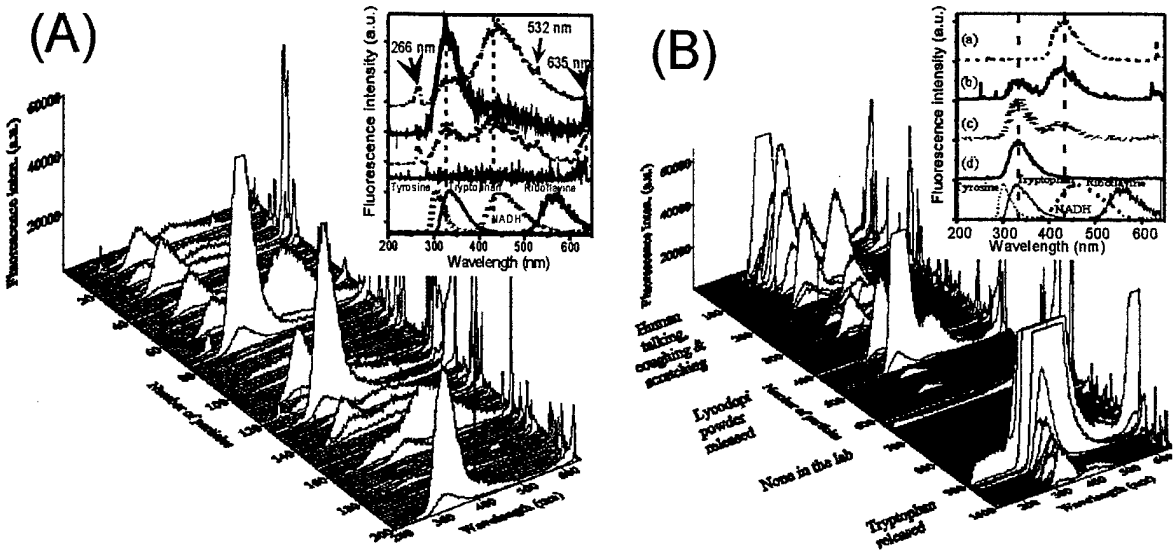

Fig. 1 (A) 200 consecutive single-shot fluorescence spectra from the ambient aerosols when a dog was wandering and being petted around. The typical spectral lineshapes are shown in the inset with the spectra from four of the primary fluorophors in biological aerosols. (B) 1000 consecutive single-shot fluorescence spectra from the ambient aerosols at the disturbance of human talking, coughing, and scratching, as well as at the releasing of some well-known aerosols. The observed spectral lineshapes from human beings are indicated as (a), (b), (c), and (d) in the inset with the spectra from four of the primary fluorophors in biological aerosols. Both the spectra from dog and human being are composed of two peaks in the UV and blue region around the similar wavelengths, but the width of the blue peak from dog is much broader, and with 5 nm blue-shift for the UV peak.

clusters with the corresponding experimental TAOS results. Such comparisons were excellent but suggested to us the need to extend the angular range for both the θ and ϕ. The TAOS data taken in the near- forward and near-backward directions, should provide, in principle, information on the cluster size and shape, the surface roughness, and possibility of the index of refraction of the primary particles. TAOS provides more physical description of the particle while fluorescence provides optical or electronic information of particle. By combining the TAOS distribution with the fluorescence spectrum from the SAME particle should produce a more stringent set of requirements that decision for classifying aerosols can be made. For example *B. subtillis* and cigarette smoke essentially have the same fluorescence spectra, but their physical size and shape being vastly different, would have generated different TAOS distributions for the bacterial cell clusters and soot.

3) We introduced a not-so-well known multi-channel detector, a 32-anode photomultiplier tube (PMT), to the field of fluorescence spectroscopy that require the detector to have single-photon sensitivity. We interested Vtech (Andover, MA) to design and build the necessary interface electronics so that the photo-electrons associated with the single laser-shot fluorescence spectra (spread from 250 nm to 700 nm and detected by 32 spectral bands) can be sample & hold until the computer was readily to receive the D-to-A data from the 32 anodes. We found that the sensitivity of the 32-anode PMT was

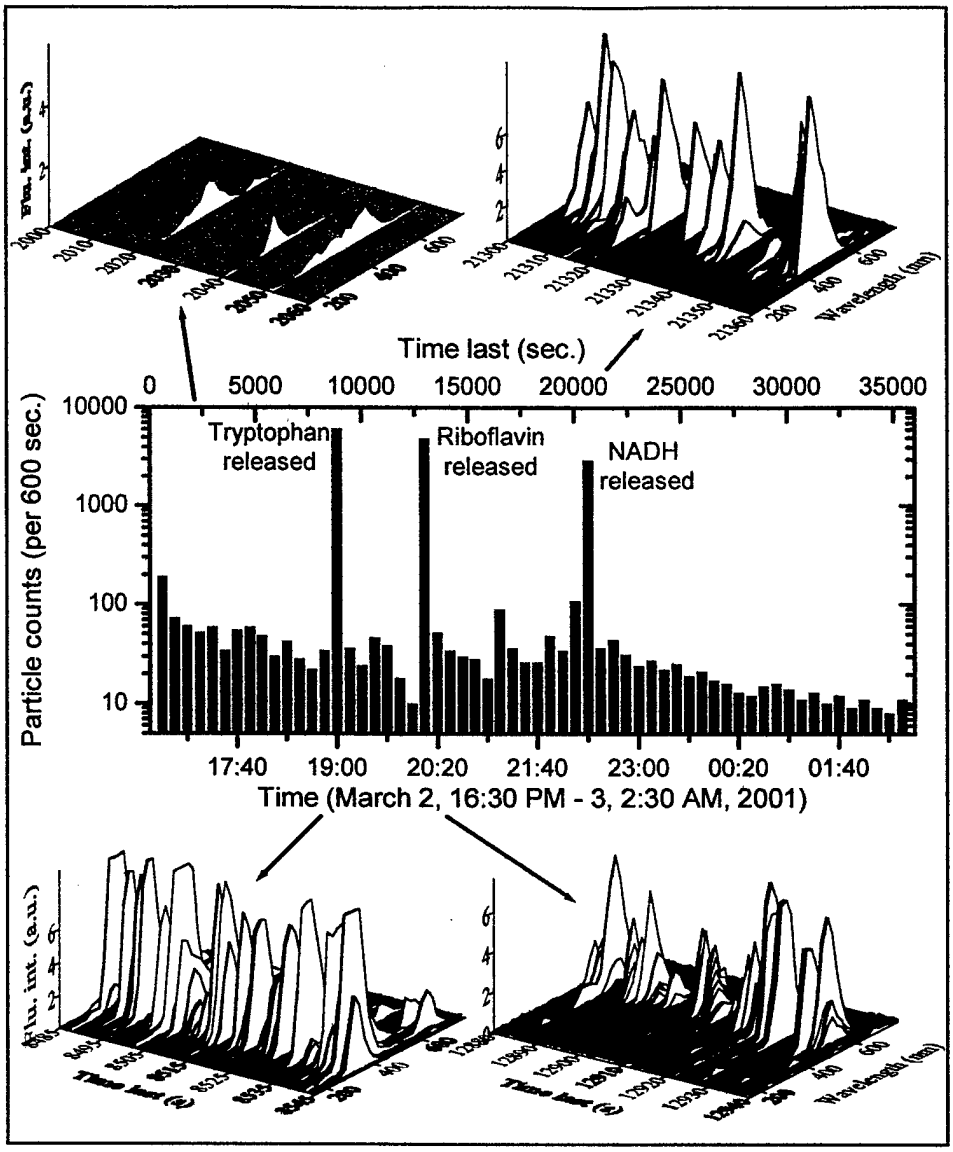

Fig. 2 The histogram of particle number (fluorescence spectra) captured by the instrument within every 10 minutes (from March 2, 16:30 pm to March 3, 2:30 am) by monitoring the ambient air in the laboratory with occasionally about 10 seconds releasing of tryptophan, riboflavine, and NADH for each sample. The four three dimensional spectra demonstrate the observed fluorescence at four time periods within 1 minute.

comparable to an "intensified CCD" detector (ICCD). However, both detectors have single-photon sensitivity. The ICCD detector that resolves the 250-700 nm spectral coverage with 1024 pixels, whereas the 32-anode PMT only resolves this coverage with 32 bands. Because the bio-aerosol fluorescence bands are so broad, the 32-bands seem adequate. However, there exist a tremendous speed advantage with the 32-anode PMT which can record fluorescence spectra at 1.4 kHz whle the ICCD record florescence spectra at 25 Hz. In a dense aerosol situation, the speed advantage of the 32-anode PMT is essential. This speed advantage allowed us to detect minority aerosols (*B. subtillis*) in

the presence of abundant amount of another type of aerosols (NADH) (see Fig.3). The time response (1 ns) of the 32-anode PMT is like that of a single-anode PMT. By keeping the 32-anode PMT behind a spectrograph, any spectral changes with time could readily be observed by recording simultaneously the time response from several different anode pins which corresponds to detecting the time response at different wavelengths (see Fig, 4).

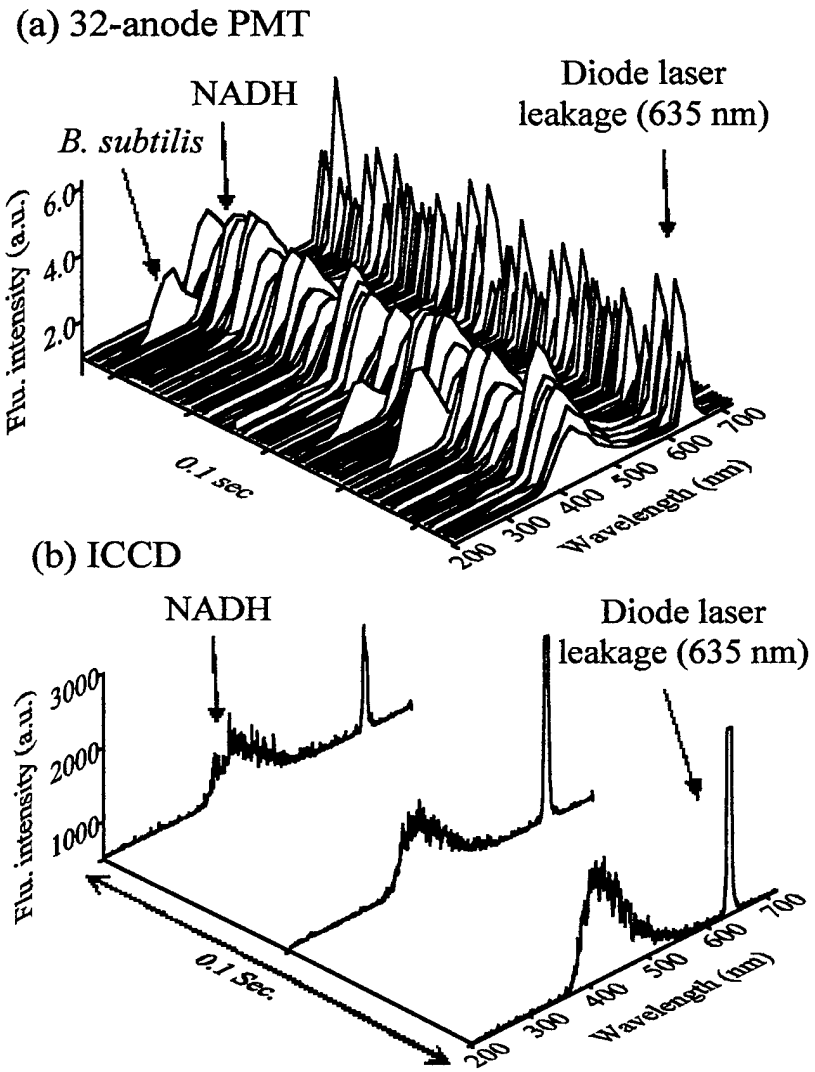

Fig. 3 Consecutive single-shot fluorescence spectra of aerosolized (≈ 5 μm in diameter) *Bacillus subtilis* bacteria and NADH mixture taken by the 32-anode PMT and ICCD-based detector systems. Within 0.1 s, the 32-anode PMT system captured 100 fluorescence spectra (can reach a repetition rate of 1.4 KHz). Because of the difference in the particle density, only 3 *Bacillus subtilis* bacteria spectra (peaked at 330 nm) were detected while dominated by the NADH aerosols (97 spectra, peaked at 450 nm). Under the same experimental conditions, the ICCD-based detector captured only 3 NADH fluorescence spectra in all. The sharp peak at 635 nm is the elastic-scattering from the diode laser.

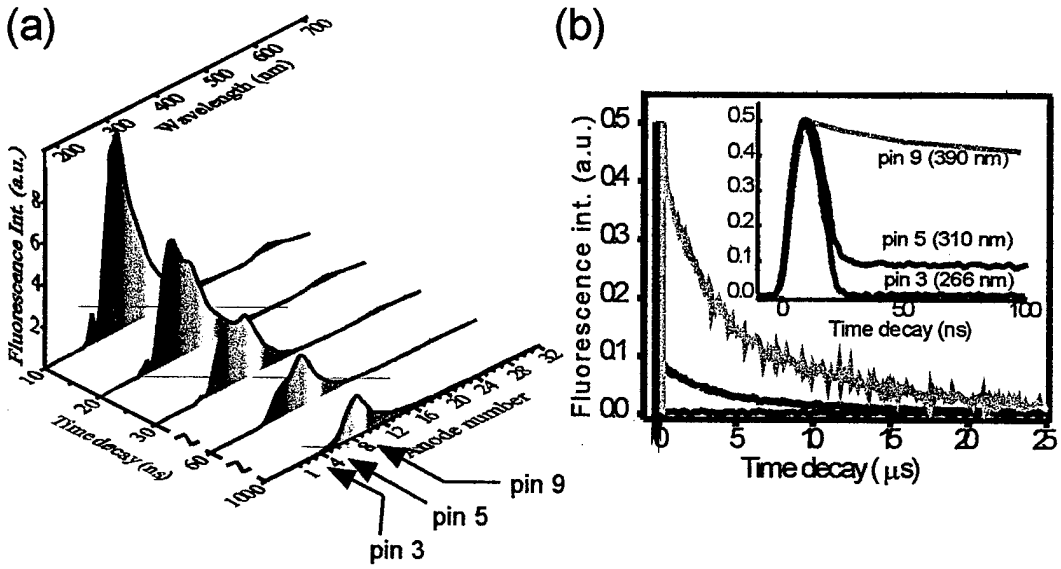

Fig. 4 (a) A set of fluorescence spectra of LiO_2 powder that were recorded (with a fixed gate interval) at different time-delay after excited by a 266-nm pulse laser (10 ns duration). (b) The temporal profiles of the laser, the 320 nm band (emission with a lifetime less than 5 ns), and the 370 nm band (emission with a lifetime about 6 μs). All three time responses were measured simultaneously by this 32-anode PMT.

During this ARO grant period, these three main results were realizable mainly because of the collaboration and cooperation with Dr. Steve Hill and Dr. Ronald Pinnick at ARL and with Dr. Jerold Bottiger at ECBC. Each of them have provided unique expertise and complementary skills as well as the joy of working together as a winning team.

LIST OF ALL PUBLICATIONS:

I. Biological Aerosols:

Steven C. Hill, Ronald G. Pinnick, Stanley Niles, Nicholas F. Fell, Jr., Yongle Pan, Jerold Bottiger, Stephen Holler, and Richard K. Chang, "Fluorescence from airborne microparticles: dependence on size, concentration of fluorophores and illumination intensity", Appl. Opt. **40**, 3005, (2001).

Yong-Le Pan, Ronald G. Pinnick, Steven C. Hill, Stanley Niles, Stephen Holler, Jerold R. Bottiger, Jean-Pierre Wolf, and Richard K. Chang, "Dynamics of Photon-induced Degradation and Fluorescence in Riboflavin Microparticles with Nanosecond UV Laser Pulse Excitation", Appl. Phys. B. 72, 449-454 (2001)

Steven C. Hill, Ronald G. Pinnick, Sranley Niles, Yong-Le Pan, Stephen Holler, Richard K. Chang, Jerold Bottiger, Bean T, Chen, Chun-Sing Orr, and Greg Feather, "Real-time measurement of fluorescence spectra from single airborne biological particles", Field Analytical Chemistry and Technology, **3**, 221, (1999).

Nicholas Fell, Ronald Pinnick, Steven Hill, Gorden Videen, Stanley Niles, Richard K. Chang, Stephen Holler, Yongle Pan, Jerold Bottiger and Burt Bronk, "Concentration, Size, and Excitation Power Effects on Fluorescence from Microdroplets and Microparticles Containing Tryptophan and Bacteria", in Air Monitoring and Detection of Chemical and Biological Agents, Joseph Leonelli, Mark L. Althouse, Editors, Proceedings of SPIE **3533**, 52 (1999).

Ronald G. Pinnick, Steven C. Hill, Paul Nachman, Gorden Videen, Gang Chen and R.K. Chang "Aerosol Fluorescence Spectrum Analyzer for Measurement of Single Micron-sized Airborne Biological Particles," Aerosol Science and Technology **28**, 95 (1998).

Yongle Pan, Stephen Holler, Richard K. Chang, Steven Hill, Ronald Pinnick, Stanley Niles, and Jerold Bottiger, "Single-Shot Fluorescence Spectra of Individual Micron-Sized Bio-Aerosols Illuminated by a 351 nm or 266 nm UV-Laser, Opt. Lett. **24**, 116, (1999).

Yongle Pan, Stephen Holler, Richard K. Chang, Steven Hill, Ronald Pinnick, Stanley Niles, Jerold Bottiger, and Burt V. Bronk, "Real-time detection and characterization of individual flowing airborne biological particles: fluorescence spectra and elastic scattering measurements," in Air Monitoring and Detection of Chemical and Biological Agents II, Joseph Leonelli, Mark L. Althouse, Editors, Proceedings of SPIE **3855**, 117-125 (1999).

II. Two-dimensional Angular Optical Scattering (TAOS)

Stephen Holler, Jean-Claude Auger, Brian Stout, Yongle Pan, Jerold R. Bottiger, Richard K. Chang, and Gorden Videen, "Observations and Calculations of Light Scattering from Clusters of Spheres", Appl. Opt., **39**, 6873, (2000). (Cover of Applied Optics)

Stephen Holler, Yongle Pan, Jerold Bottiger, Steven Hill, David B. Hillis and Richard K. Chang, "Two-dimensional Angular Scattering Measurements of Single Airborne Micro-Particles", in Air Monitoring and Detection of Chemical and Biological Agents, Joseph Leonelli, Mark L. Althouse, Editors, Proceedings of SPIE **3533**, 64 (1999).

Stephen Holler, Yongle Pan, Richard K. Chang, Jerold Bottiger, Steven C. Hill, David B. Hillis, "Two-dimensional Angular Light Scattering for the Characterization of Airborne Micro-particles," Opt. Lett. **23**, 1489, (1998).

III. 32- Anode PMT

Yong-le Pan, Patrick Cobler, Alexander Potter, Tim Chou, Richard K. Chang, Ronald Pinnick, Steven Hill, and Jean-Pierre Wolf, "High-speed, High-sensitivity Aerosol Fluorescence Spectrum Detection Using a 32-anode Photomultiplier Tube Detector", Review of Scientific Instruments, **72**, 1831, (2001).

Yong-Le Pan and Richard K. Chang, "Multiple-anode PMT behaves like many detectors in one", Laser Focus World, **37 (5)**, 235-240 (2001)

IV. Enhanced Backward Directed Fluorescence

Steven C. Hill, Yong-le Pan, Stephen Holler, Richard K. Chang, Veronique Boutou, Jin Yu, Stephane Ramstein, and Jean-Pierre Wolf, "Enhanced Backward-Directed Multi-Photon-Excited Fluorescence from Dielectric Microcavities", Phys. Rev. Lett. **85**, 54-57, (2000).

V. Review of Nonlinear Optical Effects in Micro-Droplets

Mitchell Fields, Jërgen Popp, Richard K. Chang, "Nonlinear Optics in Microspheres", Progress in Optics, 41, edited by Emil Wolf (Elsevier Science Publishers, 2001), 1-97.

LIST OF ALL PARTICIPATING SCIENTIFIC PERSONNEL:

Steven Holler (earned his Ph.D from Yale University in May 2000, now working at Los Gatos Research)

Yongle Pan (Associate Professor from Fudan University, worked at Yale University as Research Associate, and now working for Physical Science Laboratory of New Mexico State University, Las Cruces, New Mexico)

Steven C. Hill (U.S. Army Research Laboratory, Adelphi, Maryland)

Ronald G. Pinnick (U.S. Army Research Laboratory, Adelphi, Maryland)

Jerold Bottiger (U.S. Army Soldier Biological Chemical Command, Aberdeen Proving Ground, Maryland)

Burt V, Bronk (U.S. Air Force Research Laboratory at the Edgewood Chemical and Biological Center, Aberdeen Proving Ground, Maryland)

Gorden Videen (U.S. Army Research Laboratory, Adelphi,, Maryland)

Stanley Niles (U.S. Army Research Laboratory, Adelphi,, Maryland)

Nicholas F. Fell, Jr. (U.S. Army Research Laboratory, Adelphi,, Maryland)

David B. Hillis (U.S. Army Research Laboratory, Adelphi,, Maryland)

REPORT OF INVENTIONS AND SUBCONTRACTS

(Pursuant to "Patent Rights" Contract Clause) (See Instructions on back)

Form Approved
OMB No. 9000-0095
Expires Aug 31, 2001

PLEASE DO NOT RETURN YOUR COMPLETED FORM TO THIS ADDRESS. RETURN COMPLETED FORM TO THE CONTRACTING OFFICER.

1.a. NAME OF CONTRACTOR/SUBCONTRACTOR	c. CONTRACT NUMBER	2.a. NAME OF GOVERNMENT PRIME CONTRACTOR	c. CONTRACT NUMBER	3. TYPE OF REPORT *(X one)*
Yale University	DAAG5-97-1-0349			a. INTERIM: X b. FINAL:
b. ADDRESS *(Include ZIP Code)*	**d. AWARD DATE** *(YYYYMMDD)*	**b. ADDRESS** *(Include ZIP Code)*	**d. AWARD DATE** *(YYYYMMDD)*	**4. REPORTING PERIOD** *(YYYYMMDD)*
P.O. Box 208337 New Haven, CT 06520	1997/08/01			a. FROM 1997/08/01 b. TO 2001/05/31

SECTION I - SUBJECT INVENTIONS

5. "SUBJECT INVENTIONS" REQUIRED TO BE REPORTED BY CONTRACTOR/SUBCONTRACTOR *(If "None," so state)*

NAME(S) OF INVENTOR(S) *(Last, First, Middle Initial)* a.	TITLE OF INVENTION(S) b.	DISCLOSURE NUMBER, PATENT APPLICATION SERIAL NUMBER OR PATENT NUMBER c.	ELECTION TO FILE PATENT APPLICATIONS *(X)* d.				CONFIRMATORY INSTRUMENT OR ASSIGNMENT FORWARDED TO CONTRACTING OFFICER *(X)* e.	
			(1) UNITED STATES		(2) FOREIGN			
			(a) YES	(b) NO	(a) YES	(b) NO	(a) YES	(b) NO
None	No Inventions							

f. EMPLOYER OF INVENTOR(S) NOT EMPLOYED BY CONTRACTOR/SUBCONTRACTOR		g. ELECTED FOREIGN COUNTRIES IN WHICH A PATENT APPLICATION WILL BE FILED	
(1) (a) NAME OF INVENTOR *(Last, First, Middle Initial)*	(2) (a) NAME OF INVENTOR *(Last, First, Middle Initial)*	(1) TITLE OF INVENTION	(2) FOREIGN COUNTRIES OF PATENT APPLICATION
(b) NAME OF EMPLOYER	(b) NAME OF EMPLOYER		
(c) ADDRESS OF EMPLOYER *(Include ZIP Code)*	(c) ADDRESS OF EMPLOYER *(Include ZIP Code)*		

SECTION II - SUBCONTRACTS *(Containing a "Patent Rights" clause)*

6. SUBCONTRACTS AWARDED BY CONTRACTOR/SUBCONTRACTOR *(If "None," so state)*

NAME OF SUBCONTRACTOR(S) a.	ADDRESS *(Include ZIP Code)* b.	SUBCONTRACT NUMBER(S) c.	FAR "PATENT RIGHTS" d.		DESCRIPTION OF WORK TO BE PERFORMED UNDER SUBCONTRACT(S) e.	SUBCONTRACT DATES *(YYYYMMDD)* f.	
			(1) CLAUSE NUMBER	(2) DATE *(YYYYMM)*		(1) AWARD	(2) ESTIMATED COMPLETION

SECTION III - CERTIFICATION

7. CERTIFICATION OF REPORT BY CONTRACTOR/SUBCONTRACTOR *(Not required if: (X as appropriate))* ☐ **SMALL BUSINESS** or ☐ **NONPROFIT ORGANIZATION**

I certify that the reporting party has procedures for prompt identification and timely disclosure of "Subject Inventions," that such procedures have been followed and that all "Subject Inventions" have been reported.

a. NAME OF AUTHORIZED CONTRACTOR/SUBCONTRACTOR OFFICIAL *(Last, First, Middle Initial)*	b. TITLE	c. SIGNATURE	d. DATE SIGNED
Polmar, Suzanne K.	Director, Grant & Contract Administration		